STUDIES ON THE LEUCOCYTE CONTENT OF MILK DRAWN FROM BRUCELLA ABORTUS INFECTED UDDERS[1]

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The significance of the presence of *Brucella abortus* in the udder of the dairy cow is a matter of controversy. Certain investigators have presented evidence to show that pathological changes of the udder tissue accompany the growth of this organism within the udder. Others have contended that the growth of *Br. abortus* within the udder does not appear to produce harmful results. In a study in which leucocyte counts were made on a large number of milk samples drawn from both *Br. abortus* infected and non-infected animals, little difference was noted in the cellular content of milk from these two classes of animals. Since a number of the infected animals had a high agglutination titer for *Br. abortus* in both the blood and milk serum and regularly produced milk of a low leucocyte content, it appeared advisable to study the leucocyte content of the milk from the reacting animals in relation to the presence of this organism in the producing quarter.

REVIEW OF LITERATURE

Cooledge (1918) found the milk from udders infected with *Br. abortus* to have an average cell count of 4,800,000 per cubic centimeter, this being five times greater than the cell count of milk drawn from apparently normal udders. He found that udders artificially infected with this organism were quick to show an increase in the cellular count. From his study, he concluded that *Br. abortus* infections accounted for many of the samples

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of milk having high cellular counts as drawn from apparently normal udders. Tweed (1923) reported an average cell count of 1,558,000 per cubic centimeter for milk drawn from *Br. abortus* infected udders as compared to 626,000 per cubic centimeter for milk from non-infected animals.

Hallman, Sholl and Delez (1928), Sholl and Torrey (1931), and Runnells and Huddleson (1925) obtained evidence by histological studies of *Br. abortus* infected udders that pathological changes in the udder tissue were associated with infections of this organism. Hallman, Sholl and Delez (1928) stated:

> It is common knowledge that mastitis is of considerable clinical importance in *Bact. abortus* infected herds. Whether primary invasion of the udder by *Bact. abortus* bears any relation to this we do not know. Our researches indicate quite clearly to us that we can no longer assume that *Bact. abortus* infection of the udder is without harmful effects. We shall not be surprised if future researches reveal that it is in the udder that *Bact. abortus* assumes its greatest economic importance.

Bang and Bendixen (1931) found no visible alteration of the milk or udder to occur even when the organisms were present up to 30,000 per cubic centimeter. When present in large numbers, they found disturbances of secretion such as are seen in latent infections by other bacteria. They observed the posterior glands to be most frequently infected.

Smith *et al.* (1923) concluded that *Br. abortus* multiplied in the residual milk in the acini and ducts rather than in the udder tissue and that the presence of this organism in the udder did not result in pathological changes. Buck (1927) stated:

> The abortion bacilli, when present do not appear to produce harmful results in the udder. The multiplication of the germ in this organ seems to be merely a provision of nature for the perpetuation of the infection during those intervals when conditions are unfavorable for growth of the germs in the uterus.

Recent studies made by a number of investigators concerning the cell count of milk drawn from normal healthy udders have thrown considerable doubt upon the results reported by Cooledge

(1918) and Tweed (1923) in regard to the average leucocyte count of milk drawn from apparently normal udders. Cherrington, Hansen, and Halversen (1933) concluded that milk from normal udders usually contains less than 50,000 cells per cubic centimeter, whereas milk from animals suffering with mastitis almost invariably contains more than 100,000 per cubic centimeter. Hucker (1933) found that no quarter free from fibrotic tissue or indurations produced milk having a cell count in excess of 150,000 per cubic centimeter. He concluded that udder quarters producing milk having cell counts of more than 150,000 per cubic centimeter should be considered as suspicious, if not definitely proved to be infected with streptococci. In a study made in this laboratory, Prouty (1934) found that approximately 60 per cent of the samples having cell counts ranging from 250,000 to 500,000 per cubic centimeter contained excessive amounts of catalase, thus indicating the presence of abnormal udder condition. Based on the catalase test, 98 per cent of the samples used in the above study having cell counts ranging from 750,000 to 1,000,000 were from abnormal or mastitis udders.

METHODS

The data to be presented were obtained from the milk of 18 *Br. abortus* infected animals. All of the animals had exhibited positive blood agglutination reactions for a period of two years or longer. Milk from cows in the advanced stages of lactation, the last three months of the lactation period, or those which had just freshened was excluded from this study for the reason that such milk often has an increased leucocyte count due to physiological rather than to pathological changes in the udder.

In a number of instances pronounced evidence of the presence of streptococcic mastitis, as shown by blood-agar plate cultures, decreased hydrogen ion concentration, and increased catalase content of the milk was observed, thus accounting in part for several of the high leucocyte counts obtained.

Samples of the fore milk were collected aseptically from each quarter into sterile containers after the initial four or five streams had been discarded. They were examined immediately.

TABLE 1

Leucocyte count of milk in relation to the presence of Br. abortus and to the agglutination titer of milk and blood serum

ANIMAL NUMBER	QUARTER	AGGLUTINATION TITER		BR. ABORTUS	TIMES COUNTED	AVERAGE CELLULAR COUNT PER CUBIC CENTIMETER
		Blood	Milk			
		cc.	*cc.*			
59	R F	0.004	0.08	−	10	84,000
	R H		0.04	−	10	215,000
	L F		0.04	+	10	56,000
	L H		0.04	−	10	144,000
72	R F	0.02	Negative	−	13	91,000
	R H		Negative	−	13	1,162,000*
	L F		Negative	−	13	85,000
	L H		Negative	−	13	76,000
82	R F	0.004	Negative	−	7	661,000
	R H		Negative	−	7	86,000
	L F		0.01	−	7	7,480,000*
	L H		0.01	+	7	203,000
88	R F	0.004	0.02	+	14	225,000
	R H		0.02	+	14	283,000
	L F		0.02	+	14	246,000
	L H		0.02	+	14	2,900,000*
94	R F	0.02	Negative	−	7	80,000
	R H		Negative	−	7	68,000
	L F		Negative	−	7	114,000
	L H		Negative	−	7	76,000
97	R F	0.02	Negative	−	4	208,000
	R H		Negative	−	4	70,000
	L F		Negative	−	4	606,000
	L H		Negative	−	4	75,000
1028	R F	0.004	0.08	−	6	118,000
	R H		Negative	−	6	967,000
	L F		0.08	−	6	303,000
	L H		0.08	−	6	113,000
1030	R F	0.004	Negative	−	5	150,000
	R H		0.01	−	5	130,000
	L F		0.01	+	5	210,000
	L H		0.08	−	5	66,000
1034	R F	0.02	Negative	−	4	290,000
	R H		Negative	−	4	125,000
	L F		Negative	−	4	383,000
	L H		Negative	−	4	63,000

TABLE 1—*Concluded*

ANIMAL NUMBER	QUARTER	AGGLUTINATION TITER		BR. ABORTUS	TIMES COUNTED	AVERAGE CELLULAR COUNT PER CUBIC CENTIMETER
		Blood	Milk			
		cc.	*cc.*			
1046	R F	0.004	0.02	+	9	86,000
	R H		0.02	+	9	53,000
	L F		0.04	+	9	91,000
	L H		0.02	+	9	78,000
2035	R F	0.004	0.04	−	8	165,000
	R H		0.02	+	8	107,000
	L F		0.04	−	8	141,000
	L H		0.02	−	8	295,000
2052	R F	0.004	Negative	−	8	190,000
	R H		Negative	−	8	110,000
	L F		Negative	−	8	276,000
	L H		Negative	−	8	350,000
2053	R F	0.004	0.04	−	5	44,000
	R H		0.01	+	5	250,000
	L F		0.04	−	5	62,000
	L H		0.02	−	5	36,000
2074	R F	0.01	0.04	+	8	67,000
	R H		0.04	−	8	333,000
	L F		0.04	−	8	40,000
	L H		0.04	−	8	80,000
2075	R F	0.004	0.01	−	11	243,000
	R H		0.01	+	11	1,710,000*
	L F		0.01	+	11	178,000
	L H		0.02	−	11	200,000
2077	R F	0.004	0.08	−	3	115,000
	R H		0.04	−	3	48,000
	L F		0.04	+	3	22,000
	L H		0.04	+	3	85,000
3011	R F	0.004	0.08	−	8	402,000
	R H		0.01	+	8	385,000
	L F		0.08	−	8	352,000
	L H		0.08	−	8	148,000
3013	R F	0.004	0.08	−	7	60,000
	R H		0.02	+	7	117,000
	L F		0.08	−	7	52,000
	L H		0.02	+	7	127,000

* Mastitis caused by streptococci.

Leucocyte counts were made by the direct microscopic method developed by Prescott and Breed (1911). Newman's (1927) formula No. 2 was used in staining the preparations. Twenty fields of each preparation were counted.

The presence of *Br. abortus* in the udder was determined by culturing the aseptically drawn milk on cooked-blood-agar plates as described by Henry, Traum and Haring (1932). Typical colonies were further identified by means of cultural and serological tests. All udder quarters reported as negative in table 1 were recorded as such only after being cultured at least five times over a period of several months' duration.

The agglutination titer of both the blood and milk serum was determined by means of the rapid macroscopic method of Huddleson and Carlson (1926). Antigen secured from the Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri, was used in these determinations. A circular prepared by the Jensen-Salsbery Laboratories and enclosed with the antigen stated that the amounts of serum 0.08, 0.04, 0.02, 0.01 and 0.004 cc. correspond approximately to the following respective dilutions as used in the tube method: 1:25, 1:50, 1:100, 1:200, and 1:500.

PRESENTATION OF DATA

The average leucocyte counts of milk drawn from individual udder quarters in relation to the presence of *Br. abortus* and the agglutination titer of both the milk and blood serum are shown in table 1. Under the agglutination titer is recorded the smallest amount of serum showing complete agglutination of the antigen.

DISCUSSION OF RESULTS

Thirteen of the 18 cows used in this study were found by cultural methods to harbor *Br. abortus* in one or more quarters of the udder. Only two animals were found to be infected in all four quarters. Seven were found to eliminate *Br. abortus* from only one quarter. Twenty-one of the 72 quarters were found to be infected.

The average leucocyte count per cubic centimeter of milk from the 21 infected quarters was 355,000 as compared to 343,000

for milk from the 51 quarters that gave negative cultural findings. During the period over which this study extended animals 88, 2075, 72 and 82 each suffered from streptococcic mastitis in one quarter. Maximum leucocyte counts for milk from these respective udder quarters were 9,500,000; 3,600,000; 4,000,000 and 14,000,000 per cubic centimeter. When the cell counts of the samples of milk drawn from the left hind quarter of cow 88 and the right hind quarter of cow 2075 were excluded in computing averages, an average cell count of 145,000 was obtained for the milk samples drawn from the remaining 19 *Br. abortus* infected quarters. Likewise, when the milk from the right hind quarter of cow 72 and the left front quarter of 82 was omitted, an average cell count of 185,000 per cubic centimeter was found for the milk drawn from the 49 remaining quarters which gave negative findings when cultured for *Br. abortus*.

In a previous study of the leucocyte content of milk a large number of samples were from animals which periodic agglutination tests had shown to be abortion free. Three hundred and ninety-six samples from 14 animals gave an average cell count of 225,000 per cubic centimeter. Several of the animals of this herd, during the time in which they were under observation, showed pronounced positive evidence of mastitis in one or more udder quarters and when the milk from these quarters was omitted an average leucocyte count of 177,000 per cubic centimeter was found for the remaining samples. No significant differences, therefore, were seen to exist between the leucocyte counts of milk drawn from *Br. abortus* infected udders and of milk from animals free from this disease.

Five of the animals giving positive blood agglutination reactions produced milk in all quarters that contained no agglutinins for *Br. abortus* and in no instance was *Br. abortus* isolated from the milk of these animals. All samples of milk giving negative agglutination reactions in amounts of milk serum of less than 0.08 cc. yielded negative cultural findings for *Br. abortus*. This organism was isolated from five of the 13 quarters in which 0.04 cc. of milk serum was necessary to produce agglutination reactions and from 16 of the 22 quarters giving positive agglutination reactions in 0.02 cc. or less of milk serum.

SUMMARY AND CONCLUSIONS

A study was made of the leucocyte content of the milk from 18 abortion-infected cows in relation to the presence of *Br. abortus* within the udder.

Thirteen cows were found by cultural methods to harbor *Br. abortus* in one or more quarters of the udder. Two were found to be infected in all quarters and seven in only one quarter. Twenty-one of the 72 quarters were found to be infected.

The average leucocyte count per cubic centimeter of milk from the 21 infected quarters was 355,000 as compared to 343,000 for milk from the 51 quarters that gave negative cultural findings. Two of the *Br. abortus* infected quarters and two of the non-infected quarters produced milk of a high leucocyte count due to the presence of active streptococcic mastitis. When the samples of milk coming from these quarters were omitted in computing averages, average cell counts of 145,000 and 185,000 per cubic centimeter were obtained for the samples from the *Br. abortus* infected and non-infected quarters respectively. Similar average leucocyte counts were obtained for samples of milk from animals in an abortion free herd.

All samples of milk giving negative agglutination reactions in amounts of milk serum less than 0.08 cc. gave negative cultural findings for *Br. abortus*.

The results of the present study showed no significant differences in the leucocyte count of milk from *Br. abortus* infected udders and of milk from animals free from this disease.

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